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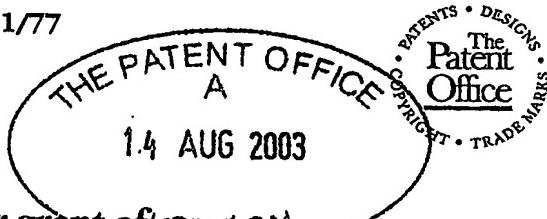
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15AU03 E830472-6 D00192
P01/7700 0.00-0319143.4**Request for grant of a patent**

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The Patent Office

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1. Your reference

N.89234 GCW

2. Patent application number

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0319143.4

3. Full name, address and postcode of the or of each applicant (underline all surnames)

 MARS, INCORPORATED
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McLean, Virginia 22101-3883
United States of America

Patents ADP number (if you know it)

7495039001

If the applicant is a corporate body, give the country/state of its incorporation

Virginia, USA

4. Title of the invention

ALLELIC VARIANTS

5. Name of your agent (if you have one)

J. A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents ADP number (if you know it)

76001

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country

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Date of filing
(day / month / year)

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Otherwise answer NO (See note d)

Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form

Description	41
Claim(s)	5
Abstract	1
Drawing(s)	4-14

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for a preliminary examination and search (Patents Form 9/77) One

Request for a substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature(s)

J. A. Kemp & Co
J.A. KEMP & CO.

Date 14 August 2003

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

WOODS, Geoffrey Corlett
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ALLELIC VARIANTSField of the invention

The present invention relates to the diagnosis and treatment of IgA-related disorders in animals, such as gastrointestinal, skin and respiratory disease, and to novel polynucleotides and polypeptides.

Background of the invention

Dogs of the German shepherd breed are particularly susceptible to a number of inflammatory and immune-mediated alimentary diseases, including small intestinal bacterial overgrowth and inflammatory bowel disease (IBD). German shepherd dogs (GSD) have also been reported to have a relative IgA deficiency, on the basis of reduced concentrations of serum IgA as compared to control populations. Similar differences in IgA concentration have been reported in tears, duodenal juice, duodenal explant culture media and faeces of this breed when compared with other breeds of dog. A reduced concentration of IgA has been found in the duodenal juice from GSD with small intestinal bacterial overgrowth compared with normal dogs, and tissue culture supernatants from twenty-four hour duodenal explants from GSD with chronic diarrhoea produce less IgA than explants derived from affected dogs of other breeds. However, in the same populations there were no differences in the numbers of IgA⁺ plasma cells or CD4⁺ T-cells within the duodenal lamina propria.

IgA plays an important role in the immune defence of mucosal sites, where it is secreted at concentrations far in excess of other immunoglobulin classes. In this context, IgA prevents colonisation and invasion by microorganisms, neutralises bacterial toxins and is involved in the elimination of antigen from the subepithelial lamina propria. The IgA molecule is of particular relevance in the immunological defence of the gastrointestinal tract, and dysfunction of mucosal immunity contributes to a range of idiopathic inflammatory bowel diseases that occur in man and other species. Multiple IgA subclasses have been identified in humans, primates and lagomorphs, whereas mice, cattle and dogs have only a single subclass. The two human subclasses (IgA₁ and IgA₂) are defined by a difference in the length of the hinge region between the CH₁ and CH₂ domains. The single IgA subclass identified in dogs has a hinge region with a predicted amino acid sequence similar to the IgA₁ subclass of humans.

Summary of the invention

The present inventors have shown that dogs possess multiple IgA allelic variants. These variants differ principally within the 5' end of the second exon of the α heavy chain gene, an area corresponding to the hinge region of the molecule. The 5 presence of an extended hinge region of IgA makes it more susceptible to cleavage by proteases. Therefore differences in hinge length and composition between variants have significant effects on the function of the IgA molecules they encode. These differences result in certain genotypes having an increased susceptibility to disease caused by pathogens that produce proteases that can cleave the hinge region.

10 Accordingly, the invention provides a method for determining susceptibility to an IgA-related disorder in an animal, the method comprising:

- a) identifying the or each IgA allelic variant present in an animal; and
- b) thereby determining whether the animal is susceptible to an IgA-related disorder. The invention further provides:

15 - a probe, primer or antibody which is capable of detecting an IgA allelic variant;

- a kit for carrying out the method of the invention comprising means for detecting an IgA allelic variant;

- a method of preparing customised food for an animal which is

20 susceptible to an IgA-related disorder, the method comprising:

(a) determining whether the animal is susceptible to an IgA-related disorder by a method of the invention; and

(b) preparing food suitable for the animal;

- a method of providing a customised animal food, comprising providing

25 food suitable for an animal which is susceptible to an IgA-related disorder to the animal, the animal's owner or the person responsible for feeding the animal, wherein the animal has been genetically determined to be susceptible to an IgA-related disorder;

- a method for identifying an agent for the treatment of an IgA-related disorder, the method comprising:

30 (a) contacting an IgA allelic variant polypeptide or a polynucleotide which encodes an IgA allelic variant with a test agent; and

(b) determining whether the agent is capable of binding to the polypeptide or modulating the activity or expression of the polypeptide or polynucleotide;

- use of a compound which is therapeutic for an IgA-related disorder in the

manufacture of a medicament for the prevention or treatment of an IgA-related disorder in an animal that has been identified as being susceptible to an IgA-related disorder by a method of the invention;

- a method of treating an animal for an IgA-related disorder, the method comprising administering to the animal an effective amount of a therapeutic compound which prevents or treats the disorder, wherein the animal has been identified as being susceptible to an IgA-related disorder by a method of the invention;

- a database comprising information relating to IgA allelic variants and optionally their association with IgA-related disorder(s);

10 - a method for determining whether an animal is susceptible to an IgA-related disorder, the method comprising:

(a) inputting data of one or more IgA allelic variant(s) present in the animal to a computer system;

(b) comparing the data to a computer database, which database comprises information relating to IgA allelic variants and the IgA-related disorder susceptibility associated with the variants; and

(c) determining on the basis of the comparison whether the animal is susceptible to an IgA-related disorder;

- a computer program comprising program code means that, when executed on a computer system, instructs the computer system to perform a method according to the invention;

- a computer program product comprising a computer-readable storage medium having recorded thereon a computer program according to the invention;

- a computer program product comprising program code means on a carrier wave, which program code means, when executed on a computer system, instruct the computer system to perform a method according to the invention;

- a computer system arranged to perform a method according to the invention comprising:

(a) means for receiving data of the one or more IgA allelic variant(s) present in the animal;

(b) a module for comparing the data with a database comprising information relating to IgA allelic variants and the IgA-related disorder susceptibility associated with the variants; and

- (c) means for determining on the basis of said comparison whether the animal is susceptible to an IgA-related disorder;
- a method of preparing customised food for an animal which is susceptible to an IgA-related disorder, the method comprising:
- 5 (a) determining whether the animal is susceptible to an IgA-related disorder by a method according to claim 23;
- (b) electropically generating a customised animal food formulation suitable for the animal;
- (c) generating electronic manufacturing instructions to control the 10 operation of food manufacturing apparatus in accordance with the customised animal food formulation; and
- (d) manufacturing the customised animal food according to the electronic manufacturing instructions;
- use of a computer system of the invention to make a customised 15 animal food product.
- an isolated polynucleotide comprising:
- (a) an IgA variant sequence that differs to SEQ ID NO: 1 at one or more polymorphic positions as defined herein;
- (b) any one of SEQ ID NO:s 3, 5, 7 or 9;
- 20 (c) a sequence that is complementary or is degenerate as a result of the genetic code to a sequence as defined in (a) or (b); or
- (d) a fragment of (a), (b) or (c) which differs to SEQ ID NO: 1 at one or more polymorphic positions as defined in claim 5 and which is at least 10 nucleotides in length; and
- 25 - a polypeptide comprising:
- (a) a sequence encoded by a polynucleotide of the invention;
- (b) any one of SEQ ID NO:s 4, 6, 8 or 10; or
- (c) a fragment of (a) or (b) which differs to SEQ ID NO: 2 at one or more polymorphic positions as defined in claim 5 and which is at least 10 amino acids in 30 length.

Brief description of the sequences

SEQ ID NO: 1 shows the polynucleotide sequence of the canine IgA α -chain, starting from the beginning of exon 1. SEQ ID NO: 2 shows the corresponding polypeptide sequence.

5 SEQ ID NO:s 3 and 4 show the polynucleotide and polypeptide sequences of variant A of the canine IgA α -chain.

SEQ ID NO:s 5 and 6 show the polynucleotide and polypeptide sequences of variant B of the canine IgA α -chain.

10 SEQ ID NO:s 7 and 8 show the polynucleotide and polypeptide sequences of variant C of the canine IgA α -chain.

SEQ ID NO:s 9 and 10 show the polynucleotide and polypeptide sequences of variant D of the canine IgA α -chain.

SEQ ID NO:s 11 to 17 show primer and probe sequences.

15 SEQ ID NO:18 shows the Genbank sequence L36871 (canine IgA α -chain polynucleotide sequence).

Brief description of the drawings

Figure 1 shows real-time RT-PCR quantification of mRNA expression in duodenal biopsies. The graph on the left (A) demonstrates the traces produced using 20 primer set 1 showing the bimodal distribution of the expression. Samples either contained a relatively 'high' (●) or 'low' (■) amounts of α -chain mRNA. The graph on the right shows the overlap of the traces when primer set 2 is used indicating that similar amounts of α -chain mRNA is present in all samples but the forward primer in set 1 does not detect a significant portion of the mRNA in some samples.

25 Figure 2 shows the four sequenced variants and their relationship to the Genbank sequence L36871. The numbering starts from the first base of exon 1 of the Genbank sequence. A single nucleotide polymorphism (C or T) (A) exists between individuals with the same variant which does not alter the predicted amino acid sequence. A variable number of CT repeats (D) are present in the intron sequence prior to the splice site of the second exon. A single base polymorphism at base 547 (T or A) (E) causes loss of the splice acceptor resulting in either long hinge variants (F to H) or 30 short hinge variants (G to H). The first exon ends at base 306 (B) and it corresponds to

the first domain of the heavy chain. Four bases in the intron sequence of all dogs sequenced do not agree with that of the Genbank sequence (C).

Figure 3 shows the mRNA sequences for the IgA variants together with the positions of the primer sets used in the real-time PCR. The positions are numbered 5 from the first base of exon one of the Genbank sequence. The position of the forward primer in set one includes the 9 base addition of variant C and D and would therefore not efficiently prime off this template. The forward primer in set 2 can amplify fragments from all variants. The difference in length of the RNA can be seen, with variant B the shortest and D the longest.

10 Figure 4 shows the predicted amino acid sequence for the IgA variants based on the sequenced mRNAs. Position A corresponds to the (A-T) polymorphism in the first exon which has no effect on the encoded amino acid. The amino acid differences are highlighted by bold text. Variant B has the shortest hinge (7 AA) and variant D the longest (13AA).

15 Figure 5 shows an alignment of mRNA encoding human IgA₁ and IgA₂. The mRNA sequences encoding the human IgA subclasses are aligned to demonstrate the position of the 39bp deletion of IgA₂ which results in the shorter hinge of this subclass. In contrast to the hinge variants of dogs, this deletion does include the initial bases of the second exon but is 5 bases from the 5' end.

20

Detailed description of the invention

The primary functional role of IgA antibodies is to protect epithelial surfaces from infectious agents. Therefore, the principal sites of IgA synthesis and secretion are the gut, the respiratory epithelium, the lactating breast and other exocrine glands such as 25 the salivary and tear glands. IgA antibodies are selectively transported across epithelia into sites such as the lumen of the gut, where they neutralise toxins and viruses and block the entry of bacteria across the intestinal epithelium. IgA antibodies are also secreted in breast milk (colostrum) and transferred to the gut of newborn offspring to provide protection against disease.

30

The IgA molecule is composed of two light chains and two heavy (α) chains. The heavy chain is divided into one variable domain (V_H) and three constant domains (C_{H1} , C_{H2} and C_{H3}). The heavy chain also comprises a flexible stretch of polypeptide chain known as the hinge region, which is located between the C_{H1} and C_{H2} domains. The flexibility of the hinge region is required to allow both arms of the antibody

molecule to bind sites that are different distances apart, and is also required for interaction with antibody-binding proteins that mediate immune effector mechanisms.

The present inventors have identified four novel allelic variants (allotypes) of canine IgA. These variants differ in the sequence of the α heavy chain gene, and in particular, in the coding region of the hinge. These differences result in variation in hinge length between each allelic variant. This variation is in part due to the presence of a single nucleotide polymorphism in the splice acceptor for the second exon at position 547 in relation to SEQ ID NO: 1. The presence of deoxythreonine (T) at position 547 results in a short hinge variant, such as variants A and B. The presence of deoxyadenosine (A) at position 547 abolishes the splice acceptor site, and therefore results in long hinge variants such as variants C and D. Variation in hinge length is also caused by other sequence differences in the coding region of the hinge, as discussed herein.

The presence of an extended hinge region of IgA makes it more susceptible to cleavage by proteases. Various pathogens secrete proteases that can cleave the hinge region of Ig molecules. Therefore, the presence of a long hinge variant of IgA in an animal may cause susceptibility to disease caused by such pathogens, for example gastrointestinal, skin and respiratory disease. Accordingly, the present invention provides a method for determining susceptibility to an IgA-related disorder in an animal, the method comprising:

- a) identifying the or each IgA allelic variant present in an animal; and
- b) thereby determining whether the animal is susceptible to an IgA-related disorder.

The IgA-related disorder may be any disease, condition or disorder that is associated with IgA deficiency or dysfunction. Such a disorder is typically an immune related disorder or a bacterial overgrowth disorder in any epithelium where IgA is secreted, such as in the gut, skin or respiratory epithelium. Therefore, the IgA-related disorder is typically a gastrointestinal, skin, respiratory, rheumatoid or periodontal disease. In particular, the disease may be diarrhoea, small intestinal bacterial overgrowth, inflammatory bowel disease, perianal fistulas, atopic dermatitis, pyoderma, anal furunculosis, malassezia infestans or disseminated aspergillosis.

The animal tested is typically a mammal, preferably a non-human animal, such as a dog, cat, horse, pig, cattle or sheep. The animal may be a companion animal or pet. In a preferred embodiment, the animal tested is a dog. The dog tested may be of any

breed, or may be a mixed or crossbred dog, or an outbred dog (mongrel). The dog may be of a breed which is prone to IgA deficiency, such as the German Shepherd Dog, beagle, cocker spaniel, Irish Wolfhound, rottweiler or shar pei. Alternatively the dog tested may be of a breed which is particularly susceptible to skin disease, such as the
5 shar pei, west highland white terrier, Labrador retriever, German Shepherd Dog or golden retriever, or a breed which is known to be susceptible to periodontal disease, such as maltese terriers, shih tsu, Yorkshire terriers, poodles and other small breed dogs.

In one embodiment, the dog tested is of a breed that is susceptible to gastrointestinal disease, such as the boxer, standard poodle, Labrador retriever, Golden
10 retriever or Irish Wolfhound. German Shepherd dogs (also known as Alsatians) are particularly susceptible to a number of gastrointestinal diseases. Therefore, in a preferred embodiment, the dog tested by a method of the invention is of the German Shepherd Dog (GSD) breed. In another aspect, the dog may be a crossbred or outbred dog which is the result of a combination of the German Shepherd Dog breed and one or
15 more other breeds.

In one embodiment of the invention, identification of the IgA allelic variant comprises detecting one or more polymorphisms in the hinge region of the IgA allelic variant, or a polymorphism which is in linkage disequilibrium with such a polymorphism. Preferably, such a polymorphism is at any one of the following
20 positions in relation to SEQ ID NO: 1:

- position 179 [C/T];
- position 370 [T/C];
- position 371 [T/C];
- position 372 [C/G];
- position 375 [G/T];
- positions 514 to 546 [number of CT repeats];
- position 547 [T/A];
- position 563 [A/T];
- positions 563 to 571 [deletion];
- positions 576 to 578 [addition];
- position 582 [C/T];
- position 583 [A/G];
- position 584 [T/A];
- position 592 [G/A]; or

- position 606 [G/A];

The animal is preferably tested before any symptoms of an IgA-related disorder may be detected. The test may therefore be used to detect susceptibility to disease in an animal, in order to allow prevent the development or onset of an IgA-related disorder.

5 Such preventative action may be related to medical treatment, dietary intervention or any other means of preventing or treating an IgA-related disorder as discussed herein. However, the test may also be used to aid or confirm a diagnosis of an IgA-related disorder in an animal.

10 *Detection of allelic variants*

The detection of allelic variants according to the invention may comprise contacting an IgA polynucleotide or protein of the animal with a specific binding agent for an IgA variant and determining whether the agent binds to the polynucleotide or protein, wherein binding of the agent indicates the presence of the IgA variant, and lack 15 of binding of the agent indicates the absence of the IgA variant.

The method is generally carried out *in vitro* on a sample from the animal. The sample typically comprises a body fluid and/or cells of the individual and may, for example, be obtained using a swab, such as a mouth swab. The sample may be a blood, urine, saliva, skin, cheek cell or hair root sample. The sample is typically processed 20 before the method is carried out, for example DNA extraction may be carried out. The polynucleotide or protein in the sample may be cleaved either physically or chemically, for example using a suitable enzyme. In one embodiment the part of polynucleotide in the sample is copied or amplified, for example by cloning or using a PCR based method prior to detecting the allelic variant(s).

25 In the present invention, any one or more methods may comprise determining the presence or absence of one or more IgA variants in the animal. The IgA variant is typically detected by directly determining the presence of the polymorphic sequence in a polynucleotide or protein of the animal. Such a polynucleotide is typically genomic DNA, mRNA or cDNA. The allelic variant may be detected by any suitable method 30 such as those mentioned below.

A specific binding agent is an agent that binds with preferential or high affinity to the protein or polypeptide having the allelic variant but does not bind or binds with only low affinity to other polypeptides or proteins. The specific binding agent may be a probe or primer. The probe may be a protein (such as an antibody) or an

oligonucleotide. The probe may be labelled or may be capable of being labelled indirectly. The binding of the probe to the polynucleotide or protein may be used to immobilise either the probe or the polynucleotide or protein.

Generally in the method, determination of the binding of the agent to the IgA variant can be carried out by determining the binding of the agent to the polynucleotide or protein of the animal. However in one embodiment the agent is also able to bind the corresponding wild-type sequence, for example by binding the nucleotides or amino acids which flank the allelic variant position, although the manner of binding to the wild-type sequence will be detectably different to the binding of a polynucleotide or protein containing the allelic variant.

The method may be based on an oligonucleotide ligation assay in which two oligonucleotide probes are used. These probes bind to adjacent areas on the polynucleotide which contains the allelic variant, allowing after binding the two probes to be ligated together by an appropriate ligase enzyme. However the presence of single mismatch within one of the probes may disrupt binding and ligation. Thus ligated probes will only occur with a polynucleotide that contains the allelic variant, and therefore the detection of the ligated product may be used to determine the presence of the allelic variant.

In one embodiment the probe is used in a heteroduplex analysis based system. In such a system when the probe is bound to polynucleotide sequence containing the allelic variant it forms a heteroduplex at the site where the allelic variant occurs and hence does not form a double strand structure. Such a heteroduplex structure can be detected by the use of single or double strand specific enzyme. Typically the probe is an RNA probe, the heteroduplex region is cleaved using RNAase H and the allelic variant is detected by detecting the cleavage products.

The method may be based on fluorescent chemical cleavage mismatch analysis which is described for example in PCR Methods and Applications 3, 268-71 (1994) and Proc. Natl. Acad. Sci. 85, 4397-4401 (1998).

In one embodiment a PCR primer is used that primes a PCR reaction only if it binds a polynucleotide containing the allelic variant, for example a sequence- or allele-specific PCR system, and the presence of the allelic variant may be determined by the detecting the PCR product. Preferably the region of the primer which is complementary to the allelic variant is at or near the 3' end of the primer. The presence of the allelic variant may be determined using a fluorescent dye and quenching agent-based PCR

assay such as the Taqman PCR detection system. The specific binding agent may be capable of specifically binding the amino acid sequence encoded by a variant sequence. For example, the agent may be an antibody or antibody fragment. The detection method may be based on an ELISA system. The method may be an RFLP based system. This can be used if the presence of the allelic variant in the polynucleotide creates or destroys a restriction site that is recognised by a restriction enzyme.

The presence of the allelic variant may be determined based on the change which the presence of the allelic variant makes to the mobility of the polynucleotide or protein during gel electrophoresis. In the case of a polynucleotide single-stranded conformation allelic variant (SSCP) or denaturing gradient gel electrophoresis (DDGE) analysis may be used. In another method of detecting the allelic variant a polynucleotide comprising the polymorphic region is sequenced across the region which contains the allelic variant to determine the presence of the allelic variant.

15 *Polynucleotides*

The invention also provides a polynucleotide which comprises an IgA variant sequence. An IgA variant sequence typically differs from SEQ ID NO: 1 (Genbank sequence L36871) at one or more of the following polymorphic positions:

- position 179 [C/T];
- position 370 [T/C];
- position 371 [T/C];
- position 372 [C/G];
- position 375 [G/T];
- positions 514 to 546 [number of CT repeats];
- position 547 [T/A];
- position 563 [A/T];
- positions 563 to 571 [deletion];
- positions 576 to 578 [addition];
- position 582 [C/T];
- position 583 [A/G];
- position 584 [T/A];
- position 592 [G/A]; or
- position 606 [G/A];

Preferably the allelic variant sequence is variant A, B, C or D as discussed herein. Accordingly, a polynucleotide of the invention preferably comprises the sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9 or a fragment thereof. The polynucleotide is typically at least 10, 15, 20, 30, 50, 100, 200 or 5 500 bases long, such as at least or up to 1kb, 10kb, 100kb, 1000 kb or more in length. The polynucleotide will typically comprise flanking nucleotides on one or both sides of (5' or 3' to) the allelic variant, for example at least 2, 5, 10, 15 or more flanking nucleotides in total or on each side. Typically, the polynucleotide will be at least 95%, preferably at least 99%, even more preferably at least 99.9% identical to the 10 polynucleotide sequences of SEQ ID NO: 3, 5, 7 or 9. Such numbers of substitutions and/or insertions and/or deletions and/or percentage identity may be taken over the entire length of the polynucleotide or over 50, 30, 15, 10 or less flanking nucleotides in total or on each side.

The polynucleotide may be RNA or DNA, including genomic DNA, synthetic 15 DNA or cDNA. The polynucleotide may be single or double stranded. The polynucleotide may comprise synthetic or modified nucleotides, such as methylphosphonate and phosphorothioate backbones or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule.

A polynucleotide of the invention may be used as a primer, for example for 20 PCR, or a probe. A polynucleotide or polypeptide of the invention may carry a revealing label. Suitable labels include radioisotopes such as ^{32}P or ^{35}S , fluorescent labels, enzyme labels or other protein labels such as biotin.

The invention also provides expression vectors that comprise polynucleotides of 25 the invention and are capable of expressing a polypeptide of the invention. Such vectors may also comprise appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Thus the coding sequence in the vector is operably linked to such elements so that they provide for expression of the coding sequence (typically in a cell). The term "operably 30 linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner.

The vector may be for example plasmid, virus or phage vector. Typically the vector has an origin of replication. The vector may comprise one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial

plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfet or transform a host cell, for example, a mammalian host cell. The vectors may also be adapted to be used *in vivo*, for example in a method of gene therapy.

- 5 Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe* *nmt1* and *adh* promoter. Mammalian promoters include the metallothionein promoter which can be induced in response to heavy metals such as cadmium. Viral promoters such as the
- 10 SV40 large T antigen promoter or adenovirus promoters may also be used. Mammalian promoters, such as β-actin promoters, may be used. Tissue-specific promoters are especially preferred. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR), the rous sarcoma virus (RSV) LTR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE
- 15 promoter, adenovirus, HSV promoters (such as the HSV IE promoters), or HPV promoters, particularly the HPV upstream regulatory region (URR).

The vector may further include sequences flanking the polynucleotide giving rise to polynucleotides which comprise sequences homologous to eukaryotic genomic sequences, preferably mammalian genomic sequences, or viral genomic sequences.

20 This will allow the introduction of the polynucleotides of the invention into the genome of eukaryotic cells or viruses by homologous recombination. In particular, a plasmid vector comprising the expression cassette flanked by viral sequences can be used to prepare a viral vector suitable for delivering the polynucleotides of the invention to a mammalian cell. Other examples of suitable viral vectors include herpes simplex viral

25 vectors and retroviruses, including lentiviruses, adenoviruses, adeno-associated viruses and HPV viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide giving rise to the polynucleotide into the host genome. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient

30 expression.

Polynucleotides of the invention may be used as a probe or primer which is capable of selectively binding to an IgA variant. Preferably the probe or primer is capable of selectively binding to the polynucleotide sequence of SEQ ID NO: 3, 5, 7 or 9. The invention thus provides a probe or primer for use in a method according to the

invention, which probe or primer is capable of selectively detecting the presence of an IgA variant. Preferably the probe is isolated or recombinant nucleic acid. It may correspond to or be antisense to the polynucleotide sequence of SEQ ID NO: 3, 5, 7 or 9. The probe may be immobilised on an array, such as a polynucleotide array.

Such primers, probes and other fragments will preferably be at least 10, preferably at least 15 or at least 20, for example at least 25, at least 30 or at least 40 nucleotides in length. They will typically be up to 40, 50, 60, 70, 100 or 150 nucleotides in length. Probes and fragments can be longer than 150 nucleotides in length, for example up to 200, 300, 400, 500, 600, 700 nucleotides in length, or even up to a few nucleotides, such as five or ten nucleotides, short of a full length polynucleotide sequence of the invention.

Homologues

Homologues of polynucleotide or protein sequences are referred to herein. Such homologues typically have at least 70% homology, preferably at least 80, 90%, 95%, 97% or 99% homology, for example over a region of at least 15, 20, 30, 100 more contiguous nucleotides or amino acids. The homology may be calculated on the basis of nucleotide or amino acid identity (sometimes referred to as "hard homology").

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* 12, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (such as identifying equivalent or corresponding sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, *supra*). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached.

5 The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

10 The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

15

The homologous sequence typically differs by at least 1, 2, 5, 10, 20 or more mutations, which may be substitutions, deletions or insertions of nucleotide or amino acids. These mutations may be measured across any of the regions mentioned above in relation to calculating homology. In the case of proteins the substitutions are preferably conservative substitutions. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

20

25

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
AROMATIC	Polar – charged	D E
		K R
AROMATIC		H F W Y

Shorter polypeptide sequences are also within the scope of the invention. For example, a fragment of a polypeptide sequence of the invention is typically at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 100, 150 or 200 amino acids in length. In particular, this aspect of the invention encompasses the situation where the polypeptide is a fragment of

5 a variant canine IgA heavy α chain which differs in amino acid sequence to a corresponding fragment of the non-variant sequence (i.e. SEQ ID NO: 1). A fragment of the variant IgA may be a Fv, F(ab') or F(ab')₂ fragment. A variant IgA fragment of the invention typically comprises the hinge region of the heavy chain.

Polypeptides of the invention may be chemically modified, for example post-
10 translationally modified. The polypeptides may be glycosylated or comprise modified amino acid residues. Such modified polypeptides fall within the scope of the term "polypeptide" of the invention.

The polypeptides, polynucleotides, vectors, cells or antibodies of the invention may be present in an isolated or substantially purified form. They may be mixed with
15 carriers or diluents which will not interfere with their intended use and still be regarded as substantially isolated. They may also be in a substantially purified form, in which case they will generally comprise at least 90%, e.g. at least 95%, 98% or 99%, of the proteins, polynucleotides, cells or dry mass of the preparation.

It is understood that any of the above features that relate to polynucleotides and
20 proteins may also be a feature of the other polypeptides and proteins mentioned herein, such as the polypeptides and proteins used in the screening and therapeutic aspects of the invention. In particular such features may be any of the lengths, modifications and vectors forms mentioned above.

25 *Detector antibodies*

The invention also provides detector antibodies that are specific for a polypeptide of the invention. A detector antibody is specific for one IgA variant, for example, variant A, B, C or D, but does not bind to any other IgA variant. The detector antibodies of the invention are for example useful in purification, isolation or screening
30 methods involving immunoprecipitation techniques.

Antibodies may be raised against specific epitopes of the polypeptides of the invention. An antibody, or other compound, "specifically binds" to a polypeptide when it binds with preferential or high affinity to the protein for which it is specific but does substantially bind not bind or binds with only low affinity to other polypeptides. A

variety of protocols for competitive binding or immunoradiometric assays to determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al*, J. Exp. Med. 158, 1211-1226, 1993). Such immunoassays typically involve the formation of complexes between the specific protein and its antibody and
5 the measurement of complex formation.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments which bind a polypeptide of the invention. Such fragments include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies.
10 Furthermore, the antibodies and fragment thereof may be chimeric antibodies, CDR-grafted antibodies or humanised antibodies.

Antibodies may be used in a method for detecting polypeptides of the invention in a biological sample (such as any such sample mentioned herein), which method comprises:

- I providing an antibody of the invention;
- 15 II incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and
- III determining whether antibody-antigen complex comprising said antibody is formed.

Antibodies of the invention can be produced by any suitable method. Means for
20 preparing and characterising antibodies are well known in the art, see for example Harlow and Lane (1988) "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. For example, an antibody may be produced by raising antibody in a host animal against the whole polypeptide or a fragment thereof, for example an antigenic epitope thereof, herein after the "immunogen". The
25 fragment may be any of the fragments mentioned herein (typically at least 10 or at least 15 amino acids long).

A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the animal's serum. The animal may therefore be inoculated
30 with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat, mouse, guinea pig, chicken, sheep or horse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

Detection kit

The invention also provides a kit that comprises means for determining the presence or absence of one or more IgA allelic variant(s) in an animal. In particular, such means may include a specific binding agent, probe, primer, pair or combination of primers, or antibody, including an antibody fragment, as defined herein which is capable of detecting or aiding detection of an IgA allelic variant. The primer or pair or combination of primers may be sequence specific primers which only cause PCR amplification of a polynucleotide sequence comprising the IgA variant to be detected, as discussed herein. The kit may also comprise a specific binding agent, probe, primer, pair or combination of primers, or antibody which is capable of detecting the absence of the allelic variant. The kit may further comprise buffers or aqueous solutions.

The kit may additionally comprise one or more other reagents or instruments which enable any of the embodiments of the method mentioned above to be carried out. Such reagents or instruments may include one or more of the following: a means to detect the binding of the agent to the allelic variant, a detectable label such as a fluorescent label, an enzyme able to act on a polynucleotide, typically a polymerase, restriction enzyme, ligase, RNase H or an enzyme which can attach a label to a polynucleotide, suitable buffer(s) or aqueous solutions for enzyme reagents, PCR primers which bind to regions flanking the allelic variant as discussed herein, a positive and/or negative control, a gel electrophoresis apparatus, a means to isolate DNA from sample, a means to obtain a sample from the individual, such as swab or an instrument

comprising a needle, or a support comprising wells on which detection reactions can be carried out. The kit may be, or include, an array such as a polynucleotide array comprising the specific binding agent, preferably a probe, of the invention. The kit typically includes a set of instructions for using the kit.

5

Screening for therapeutic agents

The present invention also relates to the use of variant IgA as a screening target for identifying therapeutic agents for the treatment of IgA-related disorders. In one embodiment the invention provides a method for identifying an agent useful for the treatment of IgA-related disorders, which method comprises contacting a variant IgA polypeptide or a polynucleotide with a test agent and determining whether the agent is capable of binding to the polypeptide or modulating the activity or expression of the polypeptide or polynucleotide. Any suitable binding assay format can be used to determine whether the IgA variant binds the test agent, such as the formats discussed below.

15 The method may be carried out *in vitro*, either inside or outside a cell, or *in vivo*. In one embodiment the method is carried out on a cell, cell culture or cell extract that comprises a variant IgA protein or polynucleotide. The cell may be any suitable cell, and is typically a cell in which the product is naturally expressed. For example, the cell 20 may be a mucosal epithelial cell such as an IgA⁺ plasma cell from the duodenal lamina propria. The method may also be carried out *in vivo* in a non-human animal which is transgenic for an IgA variant polynucleotide. The transgenic non-human animal is typically of a species commonly used in biomedical research and is preferably a laboratory strain. Suitable animals include rodents, particularly a mouse, rat, guinea 25 pig, ferret, gerbil or hamster. Most preferably the animal is a mouse.

30 The term "modulate" includes any of the ways mentioned herein in which the agent is able to modulate activity of an IgA variant polypeptide or polynucleotide. This may be determined by contacting the polypeptide or polynucleotide with the test agent under conditions that permit activity of the polypeptide or polynucleotide, and determining whether the test agent is able to modulate the activity of the polypeptide or polynucleotide.

The activity which is measured may be any of the activities which are mentioned herein, and may be the measurement of a property of the IgA variant polypeptide or polynucleotide, or an effect on a cellular component, cell or animal in which the method

is being carried out. The effect may be one that is associated with an IgA-related disorder, and may be a characteristic or symptom of an IgA-related disorder, such as any such characteristic or symptom mentioned herein.

In one embodiment the assay measures the effect of the test agent on the binding
5 between the variant IgA polypeptide or polynucleotide and another agent, such as a protease. In particular, the assay may include proteases from pathogens that are known to cause IgA-related disorders in dogs. Suitable assays in order to measure the changes in such interactions include fluorescence imaging plate reader assays, and radioligand binding assays. In the case where the activity is transcription from a gene the method
10 may comprise measuring the ability of the candidate substance to modulate transcription, for example in a reporter gene assay.

Suitable candidate agents which may be tested in the above screening methods include antibody agents, for example monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies and CDR-grafted antibodies. Furthermore,
15 combinatorial libraries, defined chemical identities, peptide and peptide mimetics, oligonucleotides and natural agent libraries, such as display libraries may also be tested. The test agents may be chemical compounds, which are typically derived from synthesis around small molecules which may have any of the properties of the agent mentioned herein. Batches of the candidate agents may be used in an initial screen of, for example,
20 ten substances per reaction, and the substances of batches which show modulation tested individually. The term 'agent' is intended to include a single substance and a combination of two, three or more substances. For example, the term agent may refer to a single peptide, a mixture of two or more peptides or a mixture of a peptide and a defined chemical entity.

25 In one aspect of the invention, the test agent is a food ingredient. Hence, the invention relates to a method of screening food ingredients to determine whether they contribute to or aggravate gastrointestinal disease in susceptible dogs, or if they prevent or alleviate gastrointestinal disease. The food ingredient may be one that is typically used in dog or other types of food, or may be a novel food ingredient.

30 The present invention also provides an agent identified by a screening method of the invention. An agent identified in the screening method of the invention may be used in the therapeutic treatment of an IgA-related disorder. Such an agent may be formulated and administered in any means or amounts as discussed below.

Treatment of IgA-related disorders

The invention provides a method of treating an animal for an IgA-related disorder, the method comprising identifying an animal which is susceptible to an IgA-related disorder by a method of the invention, and administering to the animal an effective amount of a therapeutic agent which treats the IgA-related disorder. The an IgA-related disorder may be any disease or disorder mentioned herein, and is typically a gastrointestinal, skin, respiratory disease, rheumatoid or periodontal disease. The therapeutic agent is typically a drug such as an anti-inflammatory (e.g. sulphur salasine), a corticosteroid (e.g. prednisolone), an antibiotic (e.g. amoxycillin or enrofloxacin) or a protease inhibitor (e.g. amprenavir). The therapeutic agent may be any drug known in the art that may be used to treat an IgA-related disorder, or may an agent identified by a screening method as discussed previously.

The therapeutic agent may be administered in various manners such as orally, intracranially, intravenously, intramuscularly, intraperitoneally, intranasally, intradernally, and subcutaneously. The pharmaceutical compositions that contain the therapeutic agent will normally be formulated with an appropriate pharmaceutically acceptable carrier or diluent depending upon the particular mode of administration being used. For instance, parenteral formulations are usually injectable fluids that use pharmaceutically and physiologically acceptable fluids such as physiological saline, balanced salt solutions, or the like as a vehicle. Oral formulations, on the other hand, may be solids, for example tablets or capsules, or liquid solutions or suspensions. In a preferred embodiment, the therapeutic agent is administered to the animal in its diet, for example in its drinking water or food.

The amount of therapeutic agent that is given to an animal will depend upon a variety of factors including the condition being treated, the nature of the animal under treatment and the severity of the condition under treatment. A typical daily dose is from about 0.1 to 50 mg per kg, preferably from about 0.1mg/kg to 10mg/kg of body weight, according to the activity of the specific inhibitor, the age, weight and conditions of the animal to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g.

Customised food

In one aspect, the invention relates to a customised diet for an animal that is susceptible to an IgA-related disorder. In a preferred embodiment, the customised food is for a companion animal or pet, such as a dog. Such a food may be in the form of, for 5 example, wet pet foods, semi-moist pet foods, dry pet foods and pet treats. Wet pet food generally has a moisture content above 65%. Semi-moist pet food typically has a moisture content between 20-65% and can include humectants and other ingredients to prevent microbial growth. Dry pet food, also called kibble, generally has a moisture content below 20% and its processing typically includes extruding, drying and/or 10 baking in heat. The ingredients of a dry pet food generally include cereal, grains, meats, poultry, fats, vitamins and minerals. The ingredients are typically mixed and put through an extruder/cooker. The product is then typically shaped and dried, and after 15 drying, flavours and fats may be coated or sprayed onto the dry product.

Accordingly, the present invention enables the preparation of customised food 15 suitable for an animal which is susceptible to an IgA-related disorder, wherein the customised animal food formulation comprises ingredients that prevent or alleviate IgA-related disorders, and/or does not comprise components that contribute to or aggravate 20 IgA-related disorders. Such ingredients may be any of those known in the art to prevent or alleviate an IgA-related disorder. Alternatively, such ingredients may be identified by screening methods as discussed herein. The preparation of customised animal food 25 may be carried out by electronic means, for example by using a computer system.

In one embodiment, the customised food may be formulated to alter the profile of food proteins in order to minimise the potential for secondary dietary sensitivity. The customised food may be hypoallergenic or may exclude ingredients that are poorly 25 tolerated or cause allergies, for example gluten-containing grains such as wheat, particular protein sources such as animal proteins, milk (lactose), eggs, soy, peanuts, shellfish, fruits or tree nuts.

In another embodiment, the customised food may be formulated to include 30 functional ingredients that help prevent or alleviate an IgA-related disorder. Such a functional ingredient may be a compound that stimulates immune function or protects against degradation of IgA, for example β -glucans or glutamine. Alternatively, exogenous IgA may be administered orally in the diet, for example using colostrum or eggs. These may be hyperimmunised to pathogens, such as pathogenic *E. coli* (e.g. EPEC), *Campylobacter* or *Salmonella*. The functional ingredient may help to prevent

or alleviate an IgA-related disorder by improving gut barrier function, for example probiotics or oligosaccharides. In one aspect, the customised food is formulated to prevent or alleviate IgA-related skin disease. Such food may comprise functional ingredients that improve the condition of the skin, for example vitamin C, taurine,
5 curcumin or aloe vera. One particular example of a skin support diet is described in International Application No. PCT/GB02/02538.

The present invention also relates to a method of providing a customised animal food, comprising providing food suitable for an animal which is susceptible to an IgA-related disorder to the animal, the animal's owner or the person responsible for feeding
10 the animal, wherein the animal has been determined to be susceptible to an IgA-related disorder by a method of the invention.

Bioinformatics

The sequences of the IgA variants may be stored in an electronic format, for
15 example in a computer database. Accordingly, the invention provides a database comprising information relating to IgA allelic variant sequences. The database may include further information about the allelic variant, for example the level of association of the allelic variant with an IgA-related disorder or the frequency of the allelic variant in the population. In one aspect of the invention, the database further comprises
20 information regarding the food components which are suitable and the food components which are not suitable for animals who possess a particular allelic variant of IgA.

A database as described herein may be used to determine the susceptibility of an animal to an IgA-related disorder. Such a determination may be carried out by electronic means, for example by using a computer system (such as a PC). Typically,
25 the determination will be carried out by inputting genetic data from the animal to a computer system; comparing the genetic data to a database comprising information relating to IgA allelic variants; and on the basis of this comparison, determining the susceptibility of the animal to an IgA-related disorder.

The invention also provides a computer program comprising program code
30 means for performing all the steps of a method of the invention when said program is run on a computer. Also provided is a computer program product comprising program code means stored on a computer readable medium for performing a method of the invention when said program is run on a computer. A computer program product comprising program code means on a carrier wave that, when executed on a computer

system, instruct the computer system to perform a method of the invention is additionally provided.

As illustrated in Figure 6, the invention also provides an apparatus arranged to perform a method according to the invention. The apparatus typically comprises a computer system, such as a PC. In one embodiment, the computer system comprises:

5 means 20 for receiving genetic data from the animal; a module 30 for comparing the data with a database 10 comprising information relating to IgA allelic variants; and means 40 for determining on the basis of said comparison the susceptibility of the animal to an IgA-related disorder.

10

Food manufacturing

In one embodiment of the invention, the manufacture of a customised animal food may be controlled electronically. Typically, information relating to the IgA allelic variant(s) present in an animal may be processed electronically to generate a customised animal food formulation. The customised animal food formulation may then be used to generate electronic manufacturing instructions to control the operation of food manufacturing apparatus. The apparatus used to carry out these steps will typically comprise a computer system, such as a PC, which comprises means 50 for processing the nutritional information to generate a customised animal food formulation; means 60 for generating electronic manufacturing instructions to control the operation of food manufacturing apparatus; and a food product manufacturing apparatus 70.

The food product manufacturing apparatus used in the present invention typically comprises one or more of the following components: container for dry pet food ingredients; container for liquids; mixer; former and/or extruder; cut-off device; cooking means (e.g. oven); cooler; packaging means; and labelling means. A dry ingredient container typically has an opening at the bottom. This opening may be covered by a volume-regulating element, such as a rotary lock. The volume-regulating element may be opened and closed according to the electronic manufacturing instructions to regulate the addition of dry ingredients to the pet food. Dry ingredients typically used in the manufacture of pet food include corn, wheat, meat and/or poultry meal. Liquid ingredients typically used in the manufacture of pet food include fat, tallow and water. A liquid container may contain a pump that can be controlled, for example by the electronic manufacturing instructions, to add a measured amount of liquid to the pet food.

In one embodiment, the dry ingredient container(s) and the liquid container(s) are coupled to a mixer and deliver the specified amounts of dry ingredients and liquids to the mixer. The mixer may be controlled by the electronic manufacturing instructions. For example, the duration or speed of mixing may be controlled. The mixed ingredients 5 are typically then delivered to a former or extruder. The former/extruder may be any former or extruder known in the art that can be used to shape the mixed ingredients into the required shape. Typically, the mixed ingredients are forced through a restricted opening under pressure to form a continuous strand. As the strand is extruded, it may be cut into pieces (kibbles) by a cut-off device, such as a knife. The kibbles are 10 typically cooked, for example in an oven. The cooking time and temperature may be controlled by the electronic manufacturing instructions. The cooking time may be altered in order to produce the desired moisture content for the food. The cooked kibbles may then be transferred to a cooler, for example a chamber containing one or more fans.

15 The food manufacturing apparatus may comprise a packaging apparatus. The packaging apparatus typically packages the food into a container such as a plastic or paper bag or box. The apparatus may also comprise means for labelling the food, typically after the food has been packaged. The label may provide information such as: ingredient list; nutritional information; date of manufacture; best before date; weight; 20 and species and/or breed(s) for which the food is suitable.

The invention is illustrated by the following Examples:

Example 1

25 Materials and Methods

Sample Collection

Endoscopic biopsies of duodenal mucosa were obtained from dogs presented to the Department of Clinical Veterinary Science, University of Bristol for investigation of 30 gastrointestinal disease. Gastroduodenoscopy was performed using a GIF-XQ230 flexible video endoscope (Olympus Keymed, Southend-on-Sea, UK). Multiple mucosal biopsies were taken at the level of the caudal duodenal flexure using FB-25K biopsy forceps (Olympus Keymed). Biopsies were placed in a 1.0-ml cryotube (NUNC, Fischer Scientific Ltd., Loughborough, Leicestershire), snap frozen in liquid nitrogen and stored at -70°C.

RNA/DNA Isolation

Two endoscopic biopsies (total tissue mass 9-16mg) were added to a green Ribolyser tube (Ribolyser System, Thermo-Hybaid, Ashford, Middlesex, UK) containing 400µl of lysis buffer from the RNA isolation kit (see below) and processed 5 for 45 seconds at 6.0 m/s to homogenise the biopsies. This lysate was processed through the RNeasy Isolation System (Qiagen Ltd., Crawley, U.K.) as per the manufacturer's protocol except that the RNA was eluted in 100µl of nuclease-free waster. This procedure produces a mixture of total RNA and a significant amount of genomic DNA. A negative control of nuclease free water was passed through the 10 extraction procedure. Samples were stored at -70°C prior to use.

DNase Digestion

DNase digestion was carried out on 50µl of the RNA/DNA mix from the extraction detailed above. DNase digestion was carried out both in solution and on the 15 subsequent Qiagen column used to further purify the RNA. In-solution DNase digestion was carried out by treating 50µl of the extraction mix with 5units of Amplification Grade DNase 1 (Invitrogen Ltd.) as per the manufacturer's instructions. In order to remove any residual DNase or EDTA from the treated RNA, the solution 20 was passed through the RNeasy Isolation System (Qiagen Ltd.) a second time using the RNA clean-up protocol. A second DNase digestion was carried out on this column using the RNase-Free DNase Set (Qiagen Ltd.).

Primer Design

Primers for production of the cloned RT-PCR and PCR products were designed 25 using Primer 3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) against the Genbank sequence for canine α-chain (accession number: L36871) and are shown in Table 1. The forward primer was located in the 3' end of exon 1 and the reverse primer in the 5' end of exon 2 which together produced a 550bp DNA product and a 300bp product with cDNA. The primer and probe sequences for identification of suitable dogs 30 to clone DNA and RNA sequences for α-chain from have been used previously for the quantification of α-chain transcript from canine endoscopic duodenal mucosal biopsies and are shown in Table 1 (α-chain set 1). This original set of primers was designed such that the forward primer spanned the junction between the first and second exon. A

second set of primers were designed using Primer3 with the same probe sequence, such that the forward primer was located in the first exon (α -chain set 2). The primer and probe set was designed such that the annealing temperatures of the primers were 60°C and the probe 8-10°C higher, and that a product of between 80 and 200 bases in length would be obtained. In order to minimise primer-dimer formation, the maximum self-complementarity was 6 and the maximum 3' self-complementarity was 2.

Table 1: Primer and Probe Sequences

Primer Set	Product Length	Forward Primer (5'-3')	Reverse Primer (5'-3')	5'-Fluorophore	Probe Sequence (5'-3')	3'-Quencher
Cloned Product Primers	550bp gDNA 300bp cDNA	TGTGAAC GTGACCT GGAATG	GCATTGG AGCCTAA AAGCAG			
α -Chain Set 1	84bp cDNA	TGTGCCCT GCAAAGA TAACA	AGGGCTG GCTTCTGT AGTGA	Texas Red	GTCATCCATG TCCCTCGTGC AATGAG	BHQ-2
α -Chain Set 2	136bp cDNA	CGTCTGTG AAATGCC AAGTG	AGGGCTG GCTTCTGT AGTGA			

10 RT-PCR

Gene specific real-time RT-PCR amplification of α -chain was performed using the Platinum Quantitative RT-PCR Thermoscript™ One-Step System (Invitrogen Ltd) using 5 μ l of RNA, 4.5mM Mg²⁺ and the primers and probe at a concentration of 200nM and 100nM respectively, in a final volume of 25 μ l. No-RT reactions were made by substituting the Thermoscript enzyme mix with 2 units of Platinum Taq DNA Polymerase (Invitrogen Ltd.). The negative control from the extraction procedure, as well as a nuclease-free water control, were included with all sample runs. The RT-PCR was performed in an iCycler IQ (Bio-Rad Laboratories Ltd, Hercules, CA) with an initial incubation of 55°C for 20 minutes (α -chain), followed by 95°C for 5 minutes and then 45 cycles of 95°C for 10 seconds, 60°C for 15 seconds during which the fluorescence data were collected. All reactions were made up on ice and placed in the iCycler held at the initial incubation temperature to minimise primer-dimer formation. The threshold cycle (Ct value) was calculated as the cycle when the fluorescence of the

sample exceeded a threshold level corresponding to 10 standard deviations from the mean of the baseline fluorescence.

RT-PCR to produce the products for cloning was performed using the Platinum Quantitative RT-PCR Thermoscript™ One-Step System (Invitrogen Ltd) using 5µl of RNA as described previously. DNA amplification was performed by substituting the Thermoscript enzyme mix with 2 units of Platinum Taq DNA Polymerase (Invitrogen Ltd.) with 5µl of the RNA/DNA mix. The PCR protocol was altered for the longer products so that after the initial incubation, 45 cycles of 95°C for 15 seconds, 60°C for 30 seconds and 72°C for 30 seconds were performed followed by a final incubation of 10 72°C for 5 minutes. These products were separated using 2% agarose gel electrophoresis and appropriately sized bands were excised from the gel and then purified using the QIAquick PCR Purification Kit (Qiagen Ltd., Crawley, UK) following the manufacturer's instructions.

15 *Cloning and Sequencing of Products*

The purified PCR products were cloned using the TOPO TA Cloning kit (Invitrogen Ltd.) as per the manufacturer's instructions using chemically competent *E.Coli*. Following white/blue colony selection, positive clones were sub-cultured overnight and the plasmids were purified using the Qiagen Plasmid Mini Prep Kit 20 (Qiagen Ltd.) as per the manufacturer's instructions. At least four clones per reaction mix (e.g. DNA and RNA) were purified for each dog and were sent to the Sequencing Service (School of Life Sciences, University of Dundee, Dundee, Scotland) for sequencing. Sequence results were aligned with the Genbank sequence using Omiga 2.0 (Accelrys, Cambridge, UK).

25

Results

IgA expression

A bimodal pattern of expression of IgA mRNA from canine duodenal mucosa was previously shown (Figure 1A) using primer set 1 (Peters, I. R., C. R. Helps, R. M. 30 Batt, M. J. Day, and E. J. Hall. 2003. Quantitative real-time RT-PCR measurement of mRNA encoding alpha-chain, pIgR and J-chain from canine duodenal mucosa. *J Immunol Methods* 275:213). To confirm the quantification data, a second set of primers were designed (Table 1). Surprisingly, when primer set 2 was used, the

bimodal distribution was lost (Figure 1B). The two primer sets differ only in the position of the forward primer, which spans the junction between exon one and two, as shown in Figure 3. These results indicated that a similar amount of α -chain mRNA is present in all samples but that the forward primer in set 1 does not detect a significant portion of the mRNA in some samples. Therefore, a primer set was designed which amplified a segment of both gDNA and cDNA which encompassed this region, in order to determine whether there were any sequence differences.

Sequencing Data

Four alternate sequences for the 5'-end of the second exon of the dog alpha heavy chain gene were identified, three of which have not previously been described (Figure 2). The variants were termed A to D, with variant A being similar to the Genbank sequence and thus the variant that was detected using primer set 1. A single nucleotide polymorphism exists at position 179 which differs between individuals with the same variant but this polymorphism does not alter the encoded amino acid (Figure 4).

A major difference between the variants was the position of the splice acceptor site for the second exon. This difference in the splice acceptor site was due to a single base polymorphism at position 547, that resulted in loss of a splice acceptor site for the second exon (Figure 2). The presence of deoxyadenosine at this point resulted in coding for the mRNA from position 549 (variants C and D), whereas the presence of a deoxythreonine at this position led to coding from position 558 (variants A and B) with a transcript that is nine bases shorter.

The shortest variant was variant B which had the deoxythreonine polymorphism at position 558 but also had a nine base deletion within the 5' end of the second exon (positions 563 to 571) and three additional bases after this deletion (positions 576 to 578). This variant also had other base differences at positions 582, 583, 592 and 606 compared with the other variants, and this resulted in alteration in the predicted amino acid sequence. The major difference between variants C and D was the presence of a three base addition similar to that in variant B between positions 576 to 578, making variant D the longest of the four.

The combination of variants found in each individual dog are detailed in Table 2. These results indicate that more than one variant can be detected in some individual dogs, suggesting that heterozygous individuals exist. There is also evidence that both

variants possessed by an individual are transcribed as the sequences were detected in both gDNA and cDNA products with the exception of * and +. These were only found in the gDNA (*) and cDNA (+) products respectively. All variants were found in two or more individuals with the exception of variant D which was only found in dog 48.

5

Table 2: Variant of IgA found in each dog sequenced

Dog	Breed	Variant A	Variant B	Variant C	Variant D
34	Labrador	-	X	X (*)	-
40	GSD	-	-	X	-
41	GSD	-	-	X	-
43	Staffordshire Bull Terrier	X	X	-	-
45	Greyhound	X	-	X (+)	-
46	Crossbred	X	X	-	-
47	Border Collie	-	-	-	X
48	Cocker Spaniel	-	-	X	

Discussion

Multiple IgA subclasses have been identified in humans, primates and lagomorphs, whereas in mice, cattle and dogs only a single subclass has previously been characterised. The two human subclasses (IgA₁ and IgA₂) are defined by a difference in the length of the hinge region between the CH₁ and CH₂ domains. The single IgA subclass previously identified in dogs has a hinge region with a predicted amino acid sequence similar to the IgA₁ subclass of humans.

The presence of the extended hinge region of human IgA₁ confers greater flexibility to the immunoglobulin molecule, facilitating antigen binding, but makes it more susceptible to cleavage by protease compared with IgA₂. The shortening of the hinge region that is present in human IgA₂ is due to a 39 base pair deletion from the second exon close to the 5' end (Figure 5). This is not caused by a shift in the splice site but is due to a separate gene locus within the immunoglobulin cluster.

Of the four variants identified in canine IgA, variants A, C and D share the greatest sequence similarity, with the difference in mRNA length due to a

polymorphism in the splice acceptor. Variant B has the shortest mRNA sequence, similar to variant A, but it also has base deletions towards the 5' end of the second exon with a greater number of base polymorphisms compared with the other variants. It is therefore possible that variants A, C and D are allelic variants at one gene locus (similar 5 to human IgA₁), and that variant B is encoded within a second locus and may therefore represent a second IgA subclass (similar to IgA₂).

In the present study, two of the eight dogs tested were GSD, and only variant C was found in these dogs. All sixteen GSD analysed in a separate study (unpublished data) had 'low' expression of mRNA when tested with primer set 1, suggesting that 10 none expressed variant A. The susceptibility of GSD to disease and relative IgA deficiency therefore appears to be related to the particular IgA variant(s) that are present within this breed.

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	tcaacgtgtc tgggtgtatc gcaagggtgg acggcatctg ctactaaacc gcccaatctt	1680	
55 ccctccctaa ataaactcca tgcttgcctt aagcagcccc gtcgttccat caggccgcct	1740		
	gtctgtccat attcggggtc tgggtgtatc tgaggcagggtt gtagagctc	1789	

CLAIMS

1. A method for determining susceptibility to an IgA-related disorder in an animal, the method comprising:
 - 5 a) identifying the or each IgA allelic variant present in a sample from the animal; and
 - b) thereby determining whether the animal is susceptible to an IgA-related disorder.
 2. A method according to claim 1, wherein the animal is a dog.
 - 10 3. A method according to claim 1 or 2, wherein the IgA-related disorder is gastrointestinal, skin, respiratory, rheumatoid or periodontal disease.
 4. A method according to claim 3, wherein the disease is diarrhoea, small intestinal bacterial overgrowth, inflammatory bowel disease, perianal fistulas, atopic dermatitis, pyoderma, anal furunculosis, malassezia infestans or disseminated aspergillosis.
 - 15 5. A method according to any one of the preceding claims, wherein identification of the allelic variant comprises detecting one or more polymorphisms in the hinge region of the IgA allelic variant, or a polymorphism which is in linkage disequilibrium with such a polymorphism.
 - 20 6. A method according to claim 5, wherein the polymorphism is at any one of the following positions in relation to SEQ ID NO: 1:
 - position 179 [C/T];
 - position 370 [T/C];
 - position 371 [T/C];
 - 25 - position 372 [C/G];
 - position 375 [G/T];
 - positions 514 to 546 [number of CT repeats];
 - position 547 [T/A];
 - position 563 [A/T];
 - 30 - positions 563 to 571 [deletion];
 - positions 576 to 578 [addition];
 - position 582 [C/T];
 - position 583 [A/G];
 - position 584 [T/A];

- position 592 [G/A]; or
- position 606 [G/A];

7. A method according to any one of the preceding claims, wherein step (a) comprises contacting a polynucleotide encoding an IgA allelic variant with a specific
5 binding agent for an allelic variant and determining whether the agent binds to the polynucleotide, wherein binding of the agent to the polynucleotide indicates the presence of the allelic variant.

8. A method according to claim 7 wherein the agent is a polynucleotide which is able to bind a polynucleotide encoding the IgA allelic variant but which does
10 not bind a polynucleotide encoding a different IgA allelic variant.

9. A method according to any one of claims 1 to 6, wherein step (a) comprises contacting an IgA allelic variant polypeptide with a specific binding agent for an allelic variant and determining whether the agent binds to the polypeptide, wherein binding of the agent to the polypeptide indicates the presence of the allelic variant.

15 10. A method according to any one of claims 1 to 6 wherein the allelic variant is detected by measuring the mobility of an IgA allelic variant polypeptide or a polynucleotide encoding an IgA allelic variant during gel electrophoresis.

11. A probe, primer or antibody which is capable of detecting an IgA allelic variant.

20 12. A kit for carrying out the method of any one of claims 1 to 10 comprising means for detecting an IgA allelic variant.

13. A kit according to claim 12, comprising a probe, primer or antibody according to claim 11.

25 14. A method of preparing customised food for an animal which is susceptible to an IgA-related disorder, the method comprising:

(a) determining whether the animal is susceptible to an IgA-related disorder by a method according to any one of claims 1 to 10; and

(b) preparing food suitable for the animal.

30 15. A method according to claim 14, wherein the customised animal food comprises ingredients which prevent or alleviate an IgA-related disorder, and/or does not comprise ingredients which contribute to or aggravate an IgA-related disorder.

16. A method according to claim 15 wherein the customised animal food comprises β-glucans, glutamine, probiotics, oligosaccharides, exogenous IgA, hypoallergenic protein, hydrolysed protein, vitamin C, taurine, curcumin or aloe vera.

17. A method according to any one of claims 14 to 16, further comprising providing the food to the animal, the animal's owner or the person responsible for feeding the animal.

18. A method of providing a customised animal food, comprising providing 5 food suitable for an animal which is susceptible to an IgA-related disorder to the animal, the animal's owner or the person responsible for feeding the animal, wherein the animal has been genetically determined to be susceptible to an IgA-related disorder.

19. A method for identifying an agent for the treatment of an IgA-related disorder, the method comprising:

10 (a) contacting an IgA allelic variant polypeptide or a polynucleotide which encodes an IgA allelic variant with a test agent; and

(b) determining whether the agent is capable of binding to the polypeptide or modulating the activity or expression of the polypeptide or polynucleotide.

20. Use of a compound which is therapeutic for an IgA-related disorder in 15 the manufacture of a medicament for the prevention or treatment of an IgA-related disorder in an animal that has been identified as being susceptible to an IgA-related disorder by a method according to any one of claims 1 to 10.

21. A method of treating an animal for an IgA-related disorder, the method comprising administering to the animal an effective amount of a therapeutic compound 20 which prevents or treats the disorder, wherein the animal has been identified as being susceptible to an IgA-related disorder by a method according to any one of claims 1 to 10.

22. A database comprising information relating to IgA allelic variants and optionally their association with IgA-related disorder(s).

25 23. A method for determining whether an animal is susceptible to an IgA-related disorder, the method comprising:

(a) inputting data of one or more IgA allelic variant(s) present in the animal to a computer system;

30 (b) comparing the data to a computer database, which database comprises information relating to IgA allelic variants and the IgA-related disorder susceptibility associated with the variants; and

(c) determining on the basis of the comparison whether the animal is susceptible to an IgA-related disorder.

24. A method according to claim 23, wherein the IgA allelic variant(s) are as defined in claim 6.

25. A computer program comprising program code means for performing all the steps of claim 23 when said program is run on a computer.

5 26. A computer program product comprising program code means stored on a computer readable medium for performing the method of claim 23 when said program product is run on a computer.

10 27. A computer program product comprising program code means on a carrier wave, which program code means, when executed on a computer system, instruct the computer system to perform a method according to claim 23.

28. A computer system arranged to perform a method according to claim 23 comprising:

(a) means for receiving data of the one or more IgA allelic variant(s) present in the animal;

15 (b) a module for comparing the data with a database comprising information relating to IgA allelic variants and the IgA-related disorder susceptibility associated with the variants; and

(c) means for determining on the basis of said comparison whether the animal is susceptible to an IgA-related disorder.

20 29. A method of preparing customised food for an animal which is susceptible to an IgA-related disorder, the method comprising:

(a) determining whether the animal is susceptible to an IgA-related disorder by a method according to claim 23;

25 (b) electronically generating a customised animal food formulation suitable for the animal;

(c) generating electronic manufacturing instructions to control the operation of food manufacturing apparatus in accordance with the customised animal food formulation; and

30 (d) manufacturing the customised animal food according to the electronic manufacturing instructions.

30. A computer system according to claim 28, further comprising:

(d) means for electronically generating a customised animal food formulation suitable for the animal;

(e) means for generating electronic manufacturing instructions to control

the operation of food manufacturing apparatus in accordance with the customised animal food formulation; and

- (f) a food product manufacturing apparatus.

31. Use of a computer system as defined in claim 30 to make a customised
5 animal food product.

32. An isolated polynucleotide comprising:

- (a) an IgA variant sequence that differs to SEQ ID NO: 1 at one or more polymorphic positions as defined in claim 6;

- (b) any one of SEQ ID NO:s 3, 5, 7 or 9;

10 (c) a sequence that is complementary or is degenerate as a result of the genetic code to a sequence as defined in (a) or (b); or

(d) a fragment of (a), (b) or (c) which differs to SEQ ID NO: 1 at one or more polymorphic positions as defined in claim 6 and which is at least 10 nucleotides in length.

15 33. A polypeptide comprising:

- (a) a sequence encoded by a polynucleotide according to claim 32;

- (b) any one of SEQ ID NO:s 4, 6, 8 or 10; or

(c) a fragment of (a) or (b) which differs to SEQ ID NO: 2 at one or more polymorphic positions as defined in claim 6 and which is at least 10 amino acids in

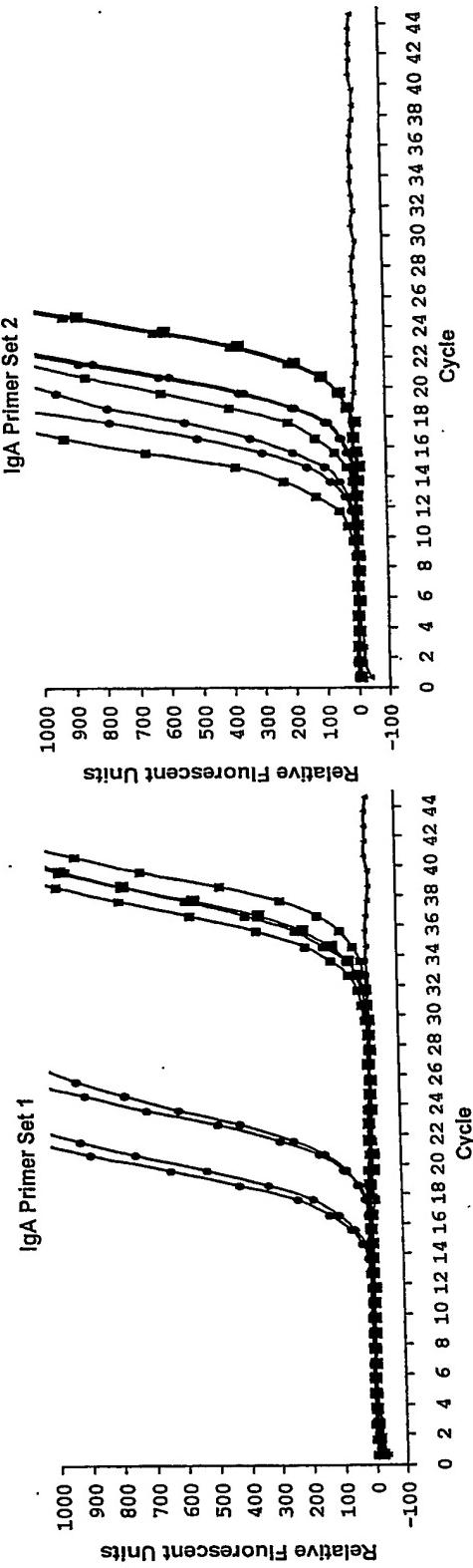
20 length.

ABSTRACT**ALLELIC VARIANTS**

5 The invention relates to a method for determining susceptibility to an IgA-related disorder in an animal, the method comprising:

- a) identifying the or each IgA allelic variant present in a sample from the animal; and
- b) thereby determining whether the animal is susceptible to an IgA-related disorder.

10 15

Figure 1

B

A

Figure 2

Figure 3

Forward Primer Set 2

A	CAGCCAGTTGACCCCTGCCAGCGCCAGTGCCTGATGACT	CGTCCTGTGA
B	CAGCCAGTTGACCCCTGCCAGCGCCAGTGCCTGATGACT	CGTCCTGTGA
C	CAGCCAGTTGACCCCTGCCAGCGCCAGTGCCTGATGACT	CGTCCTGTGA
D	CAGCCAGTTGACCCCTGCCAGCGCCAGTGCCTGATGACT	CGTCCTGTGA

Exon 1

Forward Primer Set 1

A	AATGCCAAGTGCAGCATGCTTCCAGCCCCAGCAAGGCAG	TGTCTGTGCC
B	AATGCCAAGTGCAGCATGCTTCCAGCCCCAGCAAGGCAG	TGTCTGTGCC
C	AATGCCAAGTGCAGCATGCTTCCAGCCCCAGCAAGGCAG	TGTCTGTGCC
D	AATGCCAAGTGCAGCATGCTTCCAGCCCCAGCAAGGCAG	TGTCTGTGCC

Hinge Region

A	TGCAAG-----ATAACAGTCACTCCGTGTC-----ATCCAT	GTCCTCTC
B	TGCAAG-----ATAACTGTC-----CTCATCTGTGTCCTCTC	GTCCTCTC
C	TGCAAGCTCCCTGAAGATAACTGTCATCCGTGTC-----ATCCAT	GTCCTCTC
D	TGCAAGCTCCCTGAAGATAACTGTCATCCGTGTCCTCATCCAA	GTCCTCTC

Exon 2

Reverse Primer

A	GTCGAATGAGCCCCGCTGTCACTACAGAACGCCAGCCCTC	3AGGATCTGC
B	ATGCAATGAGCCCCACCTGTCACTACAGAACGCCAGCCCTC	CGAGGATCTGC
C	GTCGAATGAGCCCCGCTGTCACTACAGAACGCCAGCCCTC	CGAGGATCTGC
D	GTCGAATGAGCCCCGCTGTCACTACAGAACGCCAGCCCTC	CGAGGATCTGC

Figure 4

A

1	10	20	30	40	50
A	VNVTNAGKDSTS VKNFPPMKAAT	TGS LYT MSSQL TL PAAQCPDDSSV K C Q			
B	VNVTNAGKDSTEV VKNFPPMKAAT	TGS LYT MSSQL TL PAAQCPDDSSV K C Q			
C	VNVTNAGKDSTS VKNFPPMKAAT	TGS LYT MSSQL TL PAAQCPDDSSV K C Q			
D	VNVTNAGKDSTS VKNFPPMKAAT	TGS LYT MSSQL TL PAAQCPDDSSV K C Q			

CH 1 Domain Hinge Region CH 2 Domain

60	70	80	90	100
A	VQHASSPSKAVSVPCK-----DNSKPC-HPCPSCNEPRLSLQKPALEDLLL LG			
B	VQHASSPSKAVSVPCK-----DN-----CPHLCPSCNEPHLSLQKPALEDLLL LG			
C	VQHASSPSKAVSVPCKAPEDNC HPCP-----SPSCNEPRLSLQKPALEDLLL LG			
D	VQHASSPSKAVSVPCKAPEDNC HPCP-----SPSCNEPRLSLQKPALEDLLL LG			

110 120 130 140 150

A	S N			
B	S N			
C	S N			
D	S N			

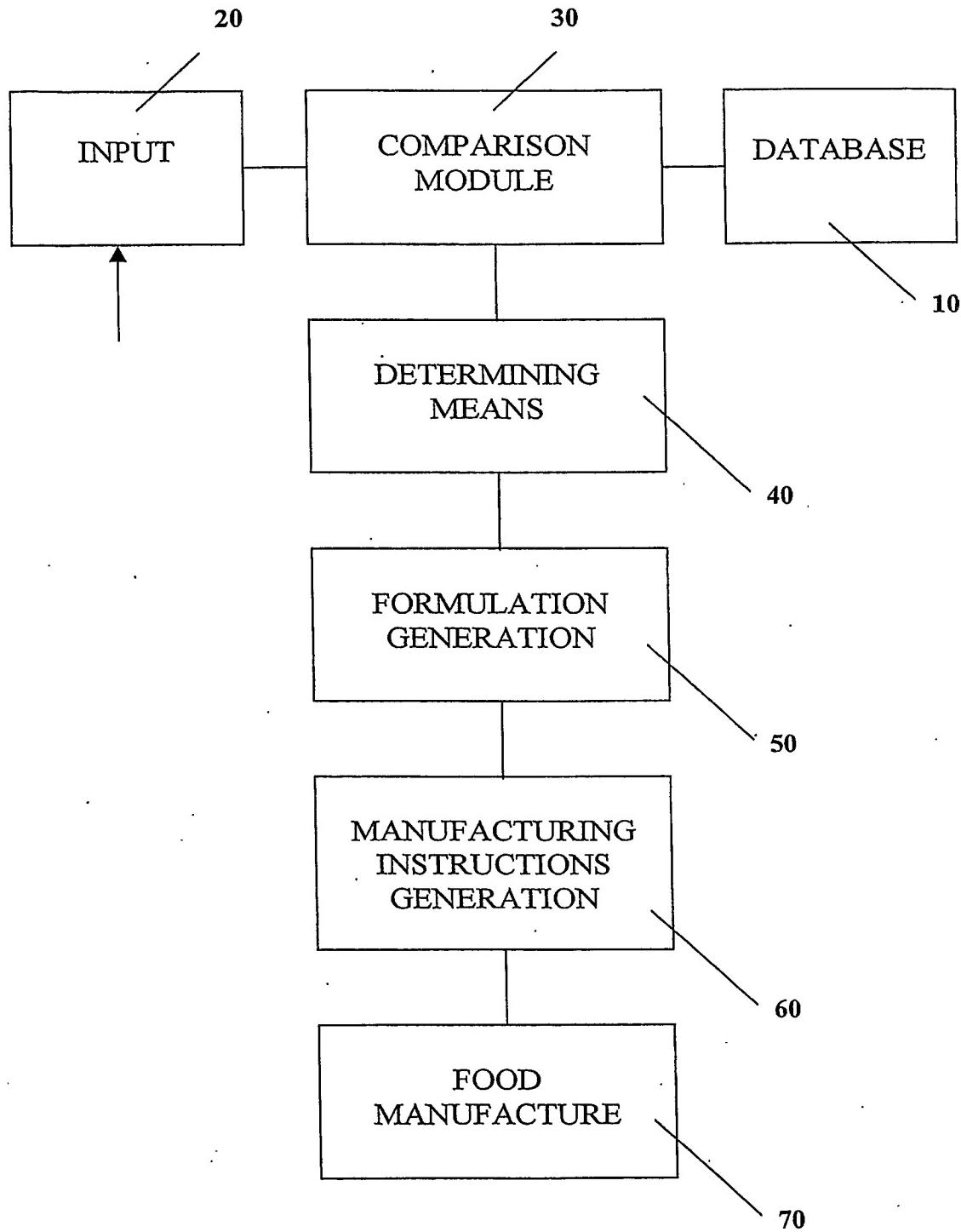
Figure 5

Exon 1 | Exon 2 ← 39bp Hinge Deletion →

Human IgA1	GGCCAGTTCCCTCAACTCCACCTACCCCATGCTCCCTCAACTCCACCTACC
Human IgA2	GGCCAGTTCCC-----

Human IgA1	CCATCTCCCTCATGCTGCCACCCCGACTGTCACTGCACTGCCACCGGACCGCCCT
Human IgA2	CCACCTCCCCCATGCTGCCACCCCGACTGTCGCTGCACTGCCACCGGACCGCCCT

Figure 6



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